# Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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## Supplemental Appendix - Table of Contents

**Study Procedures**

*Inclusion and Exclusion Criteria*

Inclusion criteria:

1) Male or female patients age ≥18 and ≤75 years of age at screening
2) Not pregnant or breast-feeding (female patients) and agrees to practice effective contraception (both sexes)
3) Documented diagnosis of HES (history of persistent eosinophilia >1500/$mm^3$ without secondary cause and evidence of end organ manifestations attributable to the eosinophilia)
4) Signs or symptoms of HES and AEC>1000/$mm^3$ on stable HES therapy for at least 1 month at the time of enrollment
5) Participation in clinical trial NCT00001406
6) Agrees to storage of samples for study

Exclusion criteria:

1) Patients with life-threatening or other serious illness or clinical manifestations deemed inappropriate for study inclusion by the PI
2) Human immunodeficiency virus (HIV) infection or other known immunodeficiency
3) Positive hepatitis B surface antigen, hepatitis C virus antibody or history of hepatitis B or C infection
4) Presence of *FIP1L1-PDGFRA* or other known imatinib-sensitive mutation
5) Diagnosis of D816V *KIT* mutation-positive systemic mastocytosis or serum tryptase level >40 ng/mL

6) Known lymphoma, hematological malignancy, advanced and metastatic solid tumors and/or patients who are under chemotherapy, radiotherapy or interleukin 2 treatment
7) Known history of allergic or anaphylactic reaction to previous antibody therapy
8) A helminth parasitic infection diagnosed within 24 weeks prior to the date informed consent is obtained
9) Acute bacterial or viral infection (patients may be enrolled once the acute infection has resolved)
10) Receipt of intravenous immunoglobulin (IVIG) within 30 days prior to the date informed consent is obtained
11) Receipt of any marketed (e.g., omalizumab) or investigational biologic within 4 months or five half-lives prior to the date informed consent is obtained, whichever is longer
12) Receipt of live attenuated vaccines 30 days prior to the date of randomization
13) Receipt of inactive/killed vaccinations (e.g., inactive influenza) are allowed provided they are not administered within 1 week before/after any study visit
14) Receipt of any investigational nonbiologic within 30 days or five half-lives prior to the date informed consent is obtained, whichever is longer
15) History of alcohol or drug abuse within 12 months prior to the date informed consent is obtained
16) Previous treatment with benralizumab (MEDI-563).

*Definition of HES clinical subtypes*

HES subtypes were defined as follows(1) :

Myeloid HES – HES with documented or presumed clonal eosinophilic involvement based on detection of a recurrent mutation associated with eosinophilic myeloid neoplasms (i.e. JAK2 mutations) or the presence of ≥4 features suggestive of a primary myeloid disorder, including dysplastic eosinophils, circulating myeloid precursors, splenomegaly, elevated serum B12 or tryptase levels, involvement of other lineages.

Lymphocytic variant HES – HES with a demonstrable clonal or phenotypically aberrant T cell population.

Overlap HES – HES with clinical manifestations restricted to a single organ system, including eosinophilic gastrointestinal disease (EGID) with peripheral eosinophilia ≥1500 cells/$mm^3$, and ANCA-negative HES meeting the American College of Rhematology criteria(2) for eosinophilic granulomatosis with polyangiitis (EGPA) but without biopsy evidence of vascultitis are included in this category.

Idiopathic HES – HES that does not meet criteria for any of the above categories and is not hereditary or due to a known secondary cause.

*Study Visits*

Screening evaluation of patients included a detailed history, physical exam and determination of eligibility. All patients were tested for *JAK2 V617F, BCR-ABL* and *FIP1L1/PDGFRA*. Patients were enrolled within 30 days of the screening visit and were randomized 1:1. Patients were evaluated daily for 3 days following the initial dose of benralizumab or placebo, weekly for 4 weeks and every 2 weeks for 8 weeks. After post-treatment reactions were noted in patient #2, subsequent patients were electively admitted to the NIH Clinical Center hospital for observation through the first three days

after drug administration, to capture these events. Patients were not pre-medicated prior to drug administration, even if they had previously exhibited a post-treatment reaction. At week 12, the baseline evaluation was repeated prior to initiation of open-label benralizumab therapy. After the first dose of open-label benralizumab, patients were again evaluated daily for 3 days, weekly for 4 weeks, and every 2 weeks for 8 weeks. Subsequent visits occurred every 4 weeks for responders and every 12 weeks for non-responders for the remainder of the trial.

A targeted history and physical examination, including AE/SAE evaluation, routine laboratory testing and collection of blood and urine samples for research studies were performed at all study visits. At baseline and weeks 12, 24, 36 and 48, electrocardiograms, transthoracic echocardiograms and pulmonary function tests were also performed. Bone marrow biopsies were performed at baseline and at week 12. Upper and lower endoscopic evaluations with tissue biopsies were performed at baseline and week 24. Bronchoscopy with bronchoavelolar lavage was performed at baseline and week 12 in one patient. Skin tissue biopsies were performed as clinically indicated.

*Study Endpoints*

The primary endpoint was a 50% reduction in peripheral blood eosinophilia on stable background therapy at 12 weeks post-initiation of study.

Secondary endpoints included: 1) Reduction in AEC at 12 weeks, 2) Frequency and severity of AEs, 3) Reduction in signs and symptoms of HES, 4) Tissue eosinophilia, 5)

Numbers of eosinophils, mast cells and their precursors in bone marrow, 6) Levels of markers of eosinophil and mast cell activation, 7) Eosinophil count and background HES therapy at the 48 week visit, and 8) drug concentrations and ADAs. Exploratory endpoints included 1) correlation between IL-5Rα levels and reduction in peripheral blood eosinophilia at 1, 4, and 12 weeks, 2) the rise in anti-tetanus antibodies at 6 weeks post-immunization and 3) the change in weight and HgbA1c levels after 12 and 24 weeks of benralizumab.

**Supplemental Methods**

*Bone marrow analysis and flow cytometry*

Bone marrow biopsies were decalcified, fixed with B-plus and embedded in paraffin according to standard protocols. Mast cells were identified by immunohistochemical staining using anti-tryptase on a Ventana Benchmark Ultra automated platform (Ventana Medical Systems, AZ, USA) following the manufacturer's instructions. Bone marrow biopsy evaluations were performed by an experienced hematopathologist (I. M.).

Bone marrow aspirates and peripheral blood samples were washed, lysed and stained with fluorochrome-conjugated antibodies against CD45, CD9, EMR-1, IL-5Rα, CD117, CD16, Siglec 8, CD69, Kappa, Lambda, CD5, CD19, CD10, CD34, CD20, CD45, CD23, CD10, CD11c, CD22, CD14, CD16, CD13, CD11b, CD38, HLA DR, CD42b, CD61, CD15, CD7, CD71, CD2, CD25, CD36, CD64, CD33, CD123, CD57, CD3, CD56, CD7, CD8, CD4, CD138, CD105 and CD235a. Multiparameter flow cytometry was performed using BD FACS Canto II system (San Jose, CA). Basophils were identified in bone marrow aspirates and peripheral blood as CD123 positive/HLA DR negative cells. Data

is presented as percentage of total viable cells. Flow cytometry data analysis was performed using FCS Express 4 (De Novo Software, Glendale, CA).

*Assessment of surface expression of IL-5Rα*

Surface expression of IL-5Rα on peripheral blood eosinophils was assessed in whole blood by flow cytometry using PE-conjugated antibody to IL-5Rα as previously described(3). Quantibrite PE beads (BD Biosciences, San Jose CA) were used to estimate the antibodies bound per cell (ABC) for CDw125-PE (clone A14; BD Biosciences, San Jose CA) per the manufacturer's instructions.

*Soluble IL-5Rα assay*

Plasma levels of soluble IL-5Rα were assessed by sandwich ELISA as previously described(3) with the following adaptations. Half-area high binding 96-well plates (#3690, Corning) were coated overnight at 4°C with anti-hIL-5Rα antibody (KM1257, provided by MedImmune) at 2 μg/mL in bicarbonate buffer (BupH Buffer #28382, ThermoFisher Scientific, Rockford IL), blocked for 1 hour at room temperature or at 4°C overnight with 1% casein in PBS (#37528, ThermoFisher Scientific, Rockford IL). Protein standards (provided by MedImmune) and thawed human plasma samples were diluted in 1% casein-PBS and incubated at room temperature for 1 hour with shaking. Plate washes were performed with Dulbecco's PBS (ThermoFisher Scientific, Rockford IL) with 0.1% Tween. Plates were incubated successively with 0.5 μg/ml biotinylated goat-anti-hIL-5Rα (BAF253, R&D, Minneapolis MN) for 1 hour at room temperature and then Amdex-streptaviden-labelled horse radish peroxidase (RPN4401V, GE Healthcare, United Kingdom) for 30 minutes, with washes in between. ELISA was developed with

KPL SureBlue peroxidase substrate at 37ºC and stopped with KPL TMB Stop Solution (Seracare, Milford MA). Plates were read on a SpectraMax i3 (Molecular Devices, San Jose CA).

*Whole blood flow cytometry on NK and Eosinophil activation*

Peripheral blood samples were stained with fluorochrome-conjugated antibodies against CD45, CD16, IL-5Rα, CD69, CD3, CD56, CD107a and CD107b. Samples were then lysed (BD FACS Lysisng buffer) and washed with PBS-BSA 1% before acquisition. Multiparameter flow cytometry was performed using BD FACS LSR II system (San Jose, CA). Eosinophils were gated as $CD45^{+}CD16^{neg}$ $SSC\text{-}A^{Hi}$ granulocytes and NK cells as $CD3^{neg}CD14^{neg}CD16CD56^{+}$. Flow cytometry data analysis was performed using FlowJo version 9.

*In vitro killing assay*

The *in vitro* killing assay was performed as previously described(4). Purified NK cells were incubated with purified autologous eosinophils in culture medium at an effector/target ratio of 5:1, in 96-well U-bottom plates at 37°C, 5%$CO_2$ in the presence or absence of 10 µg/mL MEDI-563 or isotype controls. After 4 hours, cells were stained with Annexin-V and 7-AAD (BD Biosciences). Samples were acquired and analyzed on a flow cytometer (LSRII, BD Biosciences). ADCC was measured by gating on Annexin-$V^{+}$ eosinophils, identified based on high SSC profile.

*Analysis of CD16 polymorphisms*

Patients' genotypes were determined by allele-specific PCR using primers previously described(5). Two reactions were run for each patient, one for each allele. The forward

primer (5' - TCA CAT ATT TAC AGA ATG GCA ATG G-3') was used in both reactions, while the G-specific reverse primer (5' -TCT CTG AAG ACA CAT TTC TAC TCC CTA C-3') was used to determine a valine (V) at amino acid position 176 and the T-specific reverse primer (5' -TCT CTG AAG ACA CAT TTC TAC TCC CTA A-3') was used to determine a phenylalanine (F) at position 176. The PCR reactions were performed with 100 ng of DNA, 200 nM of each primer, 200 µM dNTP, 2 mM $MgCl_2$, and 2 U of AmpliTaq polymerase (Applied Biosystems, Foster City, CA) in a 50 µl reaction volume using an Applied Biosystems Veriti 96 Well Thermocycler. The reaction started with 1) denaturation at 95 ºC for 5 min, 2) 15 cycles of touchdown PCR at 95 ºC for 30 s, annealing at 62 ºC for 30 s with the temperature decreasing by 0.5 ºC every cycle, then 3) extension at 72 ºC for 20 s. This was followed by 15-20 PCR cycles of 95 ºC for 30 s, annealing at 55 ºC for 30 s, extension at 72 ºC for 20 s with a final extension at 72 ºC for 10 min. The product of 138 bp was assayed on a 2% agarose gel (Invitrogen, Carslbad, CA). A 138 bp PCR product in the G or T allele-specific reaction indicates the presence of the valine or phenylalanine, respectively. A panel of normal donors were screened to identify homozygotes and heterozygotes as controls.

*Eosinophil Granule Protein Levels*

Suspension array multiplex immunoassay analysis of eosinophil granule proteins was performed on serum samples as previously described(6). All assays were performed in duplicate, with the final median fluorescence intensity (MFI) determined by the average from duplicate samples and concentrations determined using a standard curve of purified eosinophil granule proteins. Median fluorescence intensities were measured

using a Luminex 200 analyzer (Bio-Rad, Hercules, CA). Minimal detectable levels of serum eosinophil granule proteins were as follows: MBP (6.60 ng/ml), ECP (41.15 ng/ml), EDN (3.64 ng/ml) and EPX (4.4 ng/ml).

*Serum benralizumab and anti-drug antibody assays*

Serum samples were obtained prior to drug adminstration and every 4 weeks over the course of the trial and stored at -80ºC until analysis at PPD Laboratories. The methods were validated for this disease (HES) using serum samples from a separate cohort of HES patients treated with a variety of HES therapies, including biologics (n=30; NCT00001406). Serum concentrations of benralizumab were measured with a validated Meso Scale Discovery electrochemiluminescent (ECL) sandwich immunoassay using two noncompeting anti-benralizumab idiotype antibodies as capture and detection reagents. The lower limit of quantiation for HES serum samples was 7.72 ng/mL.

Anti-drug antibody (ADA) analyses were performed using a validated solution phase-bridging ECL method as previously described(7). Samples were measured at a 1:50 dilution using a tiered approach consisting of screening, confirmatory and titer analyses and cut points (1.09 screening, 21.1% confirmatory) statistically determined from HES patient serum samples. The method had a sensitivity of 12.5 ng/mL and provided a drug tolerance factor of approximately 500-fold using surrogate polyclonal antibody control.

*Pharmacokinetic Model for Benralizumab in HES Study*

A population pharmacokinetic (PK) model for benralizumab was originally developed by analyzing PK data from two healthy volunteer studies and four studies in patients with

asthma(8). The model was updated with data from five later phase studies in asthma (NCT01238861, CALIMA, SIRROCO, CALIMA, ZONDA and BISE). Using the same dosing schedule as in this HES trial (30 mg Q4W) and body weight and baseline eosinophil count randomly sampled from SIROCCO and CALIMA patients, PK profiles for 5,000 virtual patients were simulated using this PK model. The observed PK concentrations from HES patients were overlayed with the simulated median and 90% prediction interval (PI) to compared the observed and model predicted PK levels (Figure S7).

*Statistical Analyses.*

The Mann-Whitney U test and Wilcoxon signed-rank test (paired analyses) were used for two-sample comparison of numeric outcomes and Fisher's exact test for comparison of nominal or binary (central version) outcomes. Confidence intervals on the difference in proportions use melding so that they are compatible with Fisher's exact test(9). One-sample t-test was used to assess whether within-subject differences were significantly different from zero within each arm (E.g. effect of benralizumab or placebo). A two-sided P-value ≤0.05 was considered statistically significant for all analyses. Holm's adjusted p-values were used for some families of hypotheses (Fig 3A, Table S3+Fig S4). No multiple comparison adjustments were done for tests on AEs.

Two sensitivity analyses were performed for the primary endpoint. First, the patient who dropped out at week 6 was eliminated, and 9/10 successes (≥50% reduction in AEC from baseline to week 12) of the patients who received benralizumab were compared to

3/9 successes of patients treated with placebo (P=0.034). Second, the patient who dropped out was counted as a success to make it hardest to reject, giving 9/10 versus 4/10 (P=0.057). Finally, the secondary efficacy endpoint was analyzed using Mann-Whitney U test on the AEC at week 12 as a percentage of the baseline AEC, after conservatively replacing the missing AEC at week 12 for the placebo patient who withdrew early with 100% reduction (P=0.001).

**Supplemental Tables.**

*Table S1. Individual patient characteristics*

| Patient | Age/ Sex | HES subtype | End Organ Invovlement | Peak AEC (cells/mm$^3$) | Prior HES Therapies | Background HES Therapy | Baseline AEC (cells/mm$^3$) |
|---|---|---|---|---|---|---|---|
| 1 | 50 F | L-HES | D | 14,070 | P | P 18mg daily | 2,930 |
| 2 | 67 M | L-HES | D, C | 21,700 | P, IMAT, HU, CsA, bendamustine, brentuximab | P 20mg daily | 9,800 |
| 3 | 42 F | L-HES | D, C, R | 3,080 | P, HU | P 12.5mg daily, IFN 1mU daily | 1,160 |
| 4 | 33 F | L-HES | D, C | 5,800 | P, MTX, MMF, thalidomide, bleach baths, wraps, phototherapy | peg-IFN 90mcg SC weekly | 1,650 |
| 5 | 58 M | Idiopathic | D, R, C | 4,280 | P, IFN | P 22.5mg, | 3,940 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | peg-IFN 50mcg SC weekly | |
| 6 | 48 F | L-HES | R, G | 24,814 | P, HU, IFN | P 10mg daily, HU 1gm daily | 2,480 |
| 7 | 35 M | Overlap (EGID) | R, G | 4,400 | P, topical budesonide, swallowed F, hydroxychloroquine, sirolimus* | budesonide 9mg swallowed, F 440 BID | 2,250 |
| 8 | 54 F | Idiopathic | D, Ca, R | 10,210 | P, HU, IFN, mepolizumab** | cyclophosphamide 7mg/m$^2$ monthly | 7,250 |
| 9 | 28 F | Overlap (EGPA) + AERD | R, sinus, D | 3,530 | P, IFN | P 25mg daily, symbicort 160/4.5 BID, F 220 BID | 1,760 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 10 | 52 F | MHES (*JAK2* V617F) | Ca, N | 6,000 | P, IFN, IMAT, mepolizumab** | HU 500mg every 3 days | 1,440 |
| 11 | 53 M | Overlap (EGID) | C, D, G, R | 17,200 | P, topical budesonide | budesonide 3mg swallowed daily | 1,480 |
| 12 | 62 F | MHES (*JAK2* exon 13) | C, N | 33,000 | P, IMAT, IFN, alemtuzumab | HU 500mg 5 days a week | 1,310 |
| 13 | 37 F | Overlap (EGID) | G | 2,602 | P, elemental diet, budesonide, cromolyn | None | 1,000 |
| 14 | 23 F | Overlap (EGID) | G | 4,300 | elemental diet, dietary therapy, topical budesonide, cromolyn, | F 220 2p BID swallowed | 1,070 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | swallowed F, sirolimus | | |
| 15 | 35 F | LHES | C, M, G | 13,400 | P, FV, IFN | None | 5,620 |
| 16 | 33 F | Overlap (EGID) | G, C, sinus | 3,930 | P, IFN, swallowed F, cromolyn, topical budesonide, sirolimus*, dietary therapy | FV 220 2p BID swallowed | 1,390 |
| 17 | 74 M | Idiopathic | C, R, M | 5,400 | P, IMAT, HU, IFN, mepolizumab**, cyclophosphamide, MMF, dasatinib, MTX, alemtuzumab, cladrabine | P 30mg daily, HU 500mg daily | 2,370 |
| 18 | 31 M | Idiopathic | C, D, R, vascular | 86,180 | P, HU, IFN | peg-IFN 180mcg SC weekly | 2,350 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 19 | 44 F | Idiopathic | Ca, R, D | 11,340 | P | P 7.5mg daily | 2,830 |
| 20 | 59 M | Idiopathic | R | 26,900 | P | P 30mg daily | 21,580 |

Demographic and clinical characteristics of the enrolled patients. Peak AEC refers to the greatest reported pre-trial AEC. Prior HES therapies indicate those previously tried by patient. Background HES therapies indicate current HES medications maintained during the placebo-controlled portion of the trial. P – prednisone, IFN – interferon-$\alpha$, swallowed F – fluticasone propionate, HU – hydroxyurea, IMAT – imatinib, MTX – methotrexate, MMF – mycophenolate mofetil, CsA – cyclosporine A. *Sirolimus treatment as part of a phase I clinical trial (NCT01814059). ** High dose (750mg IV every 4 weeks) mepolizumab received as part of a phase 2 clinical trial (NCT00086658) or compassionate use program (NCT00244686). C – constitutional, R – respiratory, M – musculoskeletal, Ca – cardiac, D – dermatologic, G – gastrointestinal, N - neurologic

*Table S2. Individual patient clinical response (reduction in HES meds)*

| No. | Week 48 Status | Day 0 Medication | Week 48 Medication |
|---|---|---|---|
| 1 | Relapse | P 18 mg | P 14 mg** |
| 2 | R | P 20 mg | P 40 mg* |
| 3 | Relapse | P 12.5 mg + IFN 1 mU | P 12.5 mg + IFN 1 mU** |
| 4 | R | peg-IFN 90 mcg SC | None |
| 5 | R | P 22.5 mg + peg-IFN 50 mcg SC | None |
| 6 | Relapse | P 10 mg + HU 1 gm | P 15 mg** |
| 7 | R | budesonide 9 mg + F 220 mg 2p BID swallowed | budesonide 6 mg + F 220 mg 2p BID swallowed |
| 8 | R | CTX 70 mg/m$^2$ monthly | None |
| 9 | R | P 25 mg | P 4 mg |
| 10 | NR | HU 500 mg every 3 days | HU 500 mg every 3 days |
| 11 | R | budesonide 3 mg | None |
| 12 | NR | HU 500 mg 5 days a week | HU 500 mg 5 days a week |

| | | | |
|---|---|---|---|
| 13 | R | None | None |
| 14 | R | F 220 mg 2p BID swallowed | F 220 mg 2p BID swallowed |
| 15 | R | None | None |
| 16 | R | F 220 mg 2p BID swallowed | F 220 mg 2p BID swallowed |
| 17 | Lost to f/u | P 30 mg + HU 500 mg | P 30 mg + HU 500 mg (week 6) |
| 18 | R | peg-IFN 180 mcg SC | None |
| 19 | R | P 7.5 mg | None |
| 20 | R | P 30 mg | None |

R = clinical responder, on benralizumab therapy at week 48, NR = non-responder, off benralizumab at week 48, relapse = initial response with recurrent symptoms and eosinophilia, off benralizumab at week 48

P – prednisone, HU - hydroxyurea, IFN – interferon-$\alpha$, F – fluticasone propionate, CTX – cyclophosphamide. *Patient #2 was receiving prednisone therapy for a concomitant diagnosis of sarcoid. **Patients #1, #3, #6 were able to transiently taper their HES therapy prior to relapse: patient #1 to prednisone 5 mg daily, patient #3 to prednisone monotherapy (12.5 mg daily), and patient #3 to prednisone monotherapy (5 mg daily). Following relapse, patients #1 and #3 were restarted on their baseline medication regimens and patient #6 resumed prednisone therapy at an increased dose of 15 mg daily (without concomitant hydroxyurea).

*Table S3. Effect of benralizumab on gastrointestinal tissue eosinophilia*

| | Baseline (Pre-treatment) | | | | | | | | Week 24 (Post-benralizumab treatment) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | #6 | #7 | #11 | #13 | #14 | #15 | #16 | Median (Range) | #6 | #7 | #11 | #13 | #14 | #15 | #16 | Median (Range) |
| Proximal/Mid Esophagus | 0 | 164 | 11 | 0 | 60 | 0 | 0 | 0 (0-164) | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 (0-1) |
| Distal Esophagus | 1 | >200 | 6 | 0 | 130 | 0 | 109 | 6 (0- 200) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 (0-1) |
| Stomach | 14 | >200 | 17 | 22 | 143 | 1 | >200 | 22 (1- 220) | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 (0-1) |
| Duodenum | -- | 43 | 90 | 53 | 59 | 18 | 4 | 48 (4-90) | 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 (0-3) |
| Terminal Ileum | -- | -- | -- | 30 | 37 | 20 | 32 | 32 (20-80) | -- | -- | 1 | 0 | 0 | 0 | 0 | 0 (0-1) |
| Colon, Ascending | 82* | -- | 80* | >200 | 34 | 47 | 77 | 77 (34-200) | -- | -- | 0 | 0 | 0 | 0 | 0 | 0 (0) |
| Colon, Transverse | -- | -- | -- | 168 | 20 | 37 | 53 | 53 (20-168) | -- | -- | 0 | 0 | 0 | 0 | 0 | 0 (0-1) |
| Colon, Descending | -- | -- | -- | 134 | 10 | 33 | 37 | 37 (10-134) | -- | -- | 0 | 0 | 0 | 0 | 0 | 0 (0-1) |
| Rectosigmoid | -- | -- | -- | 157 | 9 | 33 | 26 | 33 (9-157) | -- | -- | 0 | 0 | 0 | 0 | 0 | 0 (0-1) |

Tissue eosinophils were enumerated by a pathologist and peak eosinophil counts at each anatomical segment (rows) are presented from individual patients (columns) who underwent upper endoscopy and colonoscopy at baseline and week 24 after benralizumab treatment for 6 months (randomized to drug) or 3 months (randomized to placebo, #13, #16). -- tissue not biopsied. * random colon biopsy taken.

*Table S4. Drug-Related Adverse Events (Baseline to Week 48)*

| Event | Episodes | Affected Patients (n=19) |
|---|---|---|
| **Serious Adverse Events** | | |
| Any event | 2 | 2 (10.5%) |
| Eosinophilia | 1 | 1 (5.3%) |
| Ureteral obstruction | 1 | 1 (5.3%) |
| **Grade 3 Adverse Events** | | |
| Eosinophilia | 3 | 2 (10.5%) |
| Shingles | 1 | 1 (5.3%) |
| Ureteral obstruction | 1 | 1 (5.3%) |
| | | |
| **Adverse Events** | | |
| Any Event | 79 | 17 (89.5%) |
| Headache | 6 | 6 (31.6%) |
| Serum LDH | 6 | 6 (31.6%) |
| Chills | 4 | 4 (21%) |
| Eosinophilia | 3 | 2 (10.5%) |
| Injection site reaction | 3 | 2 (10.5%) |
| Malaise | 3 | 2 (10.5%) |
| ALT increased | 2 | 2 (10.5%) |
| AST increased | 2 | 2 (10.5%) |
| Lymphopenia | 2 | 2 (10.5%) |

| | | |
|---|---|---|
| Creatinine increased | 2 | 2 (10.5%) |
| Diarrhea | 2 | 2 (10.5%) |
| Fever | 2 | 2 (10.5%) |
| Hyperuricemia | 2 | 2 (10.5%) |
| Hypophosphatemia | 2 | 2 (10.5%) |
| Pruritic rash | 2 | 2 (10.5%) |

Events that are possibly, probably and definitely related are tabulated. All serious adverse events and Grade 3 adverse events, as well as Grade 1 and Grade 2 adverse events that are reported for ≥2 patients, are presented. MedDRA lowest level terms was used (Versions 16.1 thru 20.1).

*Table S5. Characteristics of post-injection reactions*

| **Patient Number/ Arm** | **Post-injection Reaction** | **Post-injection Symptoms** | | **Serum LDH (ng/mL)** | | | |
|---|---|---|---|---|---|---|---|
| | | **BL** | **Week 12** | **BL** | | **Week 12** | |
| | | **Day 0** | **Day 84** | **Day 0** | **Day 1** | **Day 84** | **Day 85** |
| 1/Placebo | No | None | None | 280 | 233 | **247** | **320** |
| 2/Drug | Yes | Fatigue, nausea, headache | None | **226** | **408** | 166 | 163 |
| 3/Drug | Yes | Headache, fever, chills, nausea | None | **169** | **203** | 162 | 177 |
| 4/Placebo | No | None | Nausea, vomiting** | 215 | (240)* | **167** | **179** |
| 5/Placebo | No | None | None | 358 | 287 | **142** | **158** |
| 6/Drug | Yes | Headache | None | **212** | **233** | 187 | 175 |
| 7/Drug | Yes | Headache, fever, chills | None | **162** | **250** | 129 | 125 |
| 8/Placebo | No | None | None | 239 | 221 | **241** | **246** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 9/Placebo | Yes | None | Headache, chills | 208 | 191 | **202** | **216** |
| 10/Placebo | No | None | None | 253 | 236 | 255 | 270 |
| 11/Drug | Yes | Myalgia, flushing, sweating | Myalgia, chills, fever | **176** | **248** | 159 | 153 |
| 12/Drug | No | None | None | **172** | **167** | 173 | (167)* |
| 13/Placebo | No | Itching at injection site | None | 138 | 163 | **160** | **176** |
| 14/Drug | No | None | None | **115** | **142** | 108 | 114 |
| 15/Placebo | Yes | None | Chills, headache | 146 | 161 | **159** | **299** |
| 16/Drug | No | None | None | **(147)*** | **194** | 117 | 126 |
| 17/Placebo | N/A | None | NA | 253 | (NA)* | ND | ND |
| 18/Placebo | No | None | None | 140 | (160)* | **124** | **128** |
| 19/Drug | Yes | Headache, malaise, fever | None | **(210)*** | **254** | (194)* | 140 |
| 20/Drug | No | None | None | **179** | **172** | 175 | 170 |

Bold is used to signify LDH values during the frst dose of benralizumab at baseline or week 12; ( )*hemolyzed, ** symptoms attributed to pain medication given for bone marrow examination.

*Table S6. Clinical and laboratory parameters potentially associated with the development of post-treatment reactions*

| Parameter | No Reaction (n=11) | Reaction (n=8) | Unadjusted *P*-values |
|---|---|---|---|
| ***HES Clinical Subtype*** | | | 0.3365 |
| Myeloid variant | 2 | 0 | |
| Lymphocytic variant | 2 | 4 | |
| Single organ overlap | 3 | 3 | |
| Idiopathic | 4 | 1 | |
| ***Baseline HES medications*** | | | n.s. |
| Corticosteroids | 6 | 7 | |
| Interferon-$\alpha$ | 3 | 1 | |
| Hydroxyurea | 2 | 1 | |
| Cyclophosphamide | 1 | 0 | |
| None | 1 | 1 | |
| ***Laboratory parameters**** | | | |
| Absolute NK cell (cells/mm$^3$) | 132 (34-511) n=11 | 204 (88-418) n=8 | 0.2291 |
| CD69$^+$ NK (%) | 4.2 (1.8-7.3) n=6 | 7.6 (4.3-12.7) n=3 | 0.1667 |
| CD107a/b$^+$ NK (%) | 3.2 (1.2-6.7) n=6 | 3.5 (2.2-4.5) n=3 | >0.9999 |
| AEC (cells/mm$^3$) | 1,556 (420-21,580) n=11 | 2,316 (1,050-9,780) n=8 | 0.2060 |

| | | | |
|---|---|---|---|
| $CD69^+$ Eosinophils (%) | 3.4%<br>(0.2-38.4)<br>n=7 | 1.4%<br>(0.7-4.5)<br>n=4 | 0.6121 |
| IL-5Rα surface (ABC) | 1,702<br>(575-4,266)<br>n=10 | 2,620<br>(1,514-3,802)<br>n=6 | 0.1471 |
| EPX (ng/mL) | 2,018<br>(767-8,931)<br>n=11 | 2,946<br>(902-7,379)<br>n=8 | 0.2723 |
| MBP (ng/mL) | 441<br>(108-1,650)<br>n=11 | 651<br>(179-2,772)<br>n=8 | 0.5448 |
| NK:Eo ratio | 0.085<br>n=11 | 0.088<br>n=8 | 0.8404 |

*at the time of the first dose of benralizumab. Geometric means and ranges of laboratory parameters are shown. Normal ranges used at NIH Clinical Center: AEC (40 – 360 cells/$mm^3$), absolute NK cell count (126 – 729 cells/$mm^3$), EPX- eosinophil peroxidase, MBP- major basic protein

**Supplementary Figures**

*Figure S1. Patient Disposition*

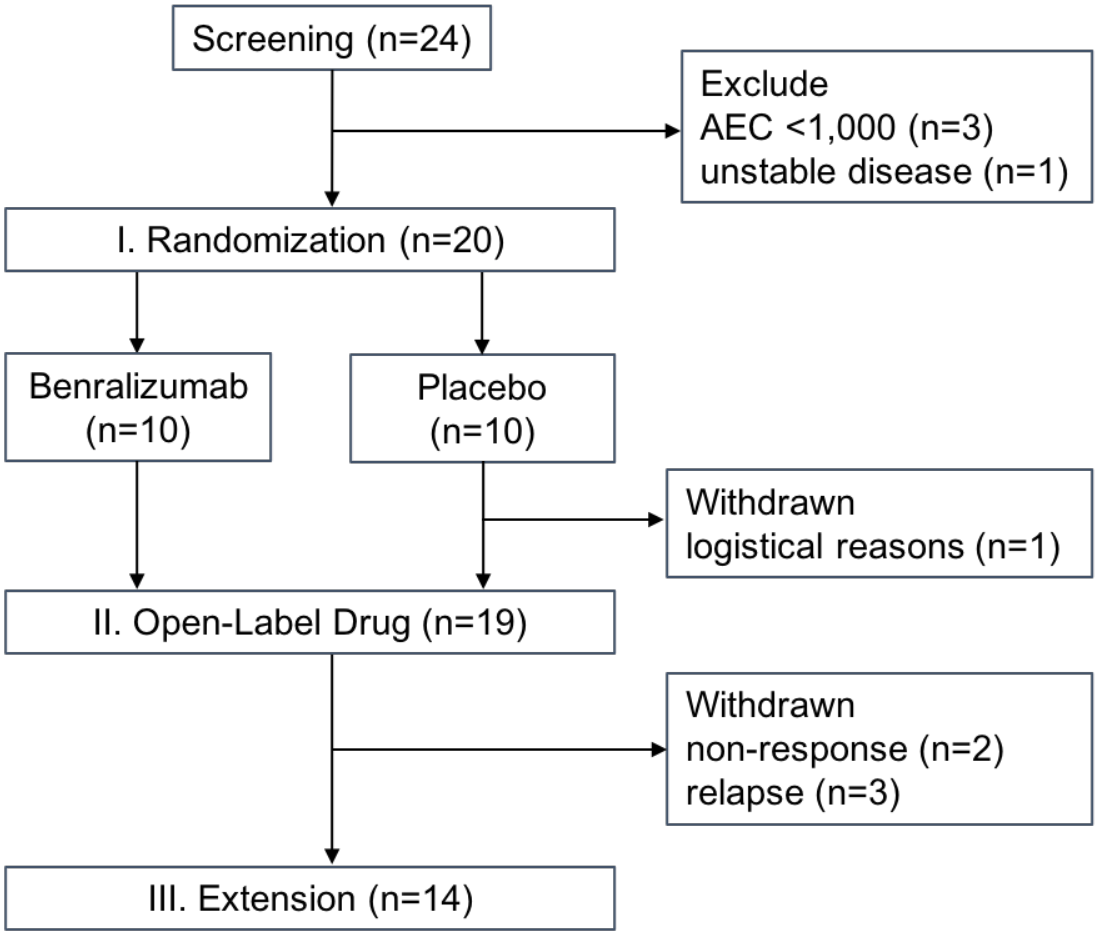
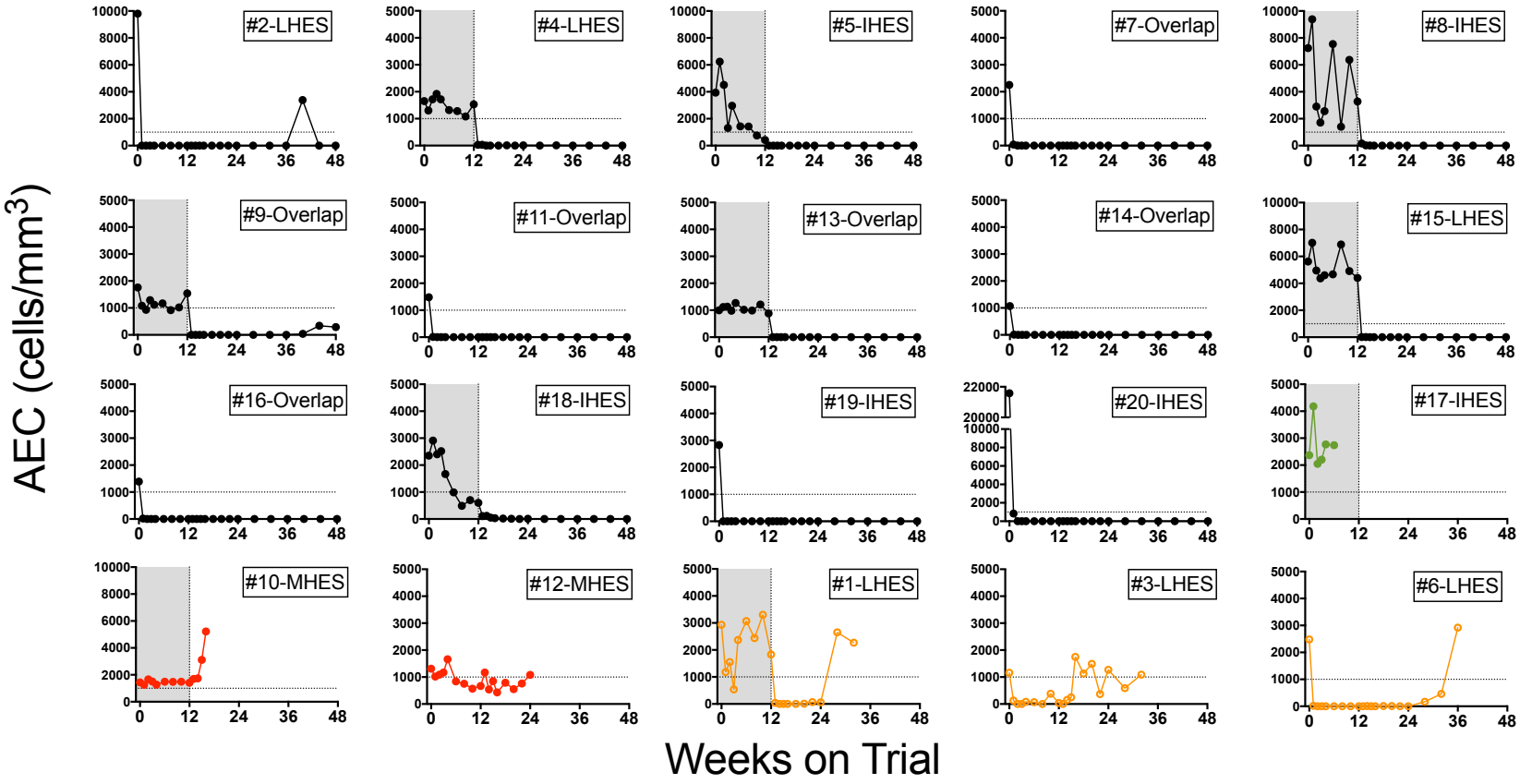
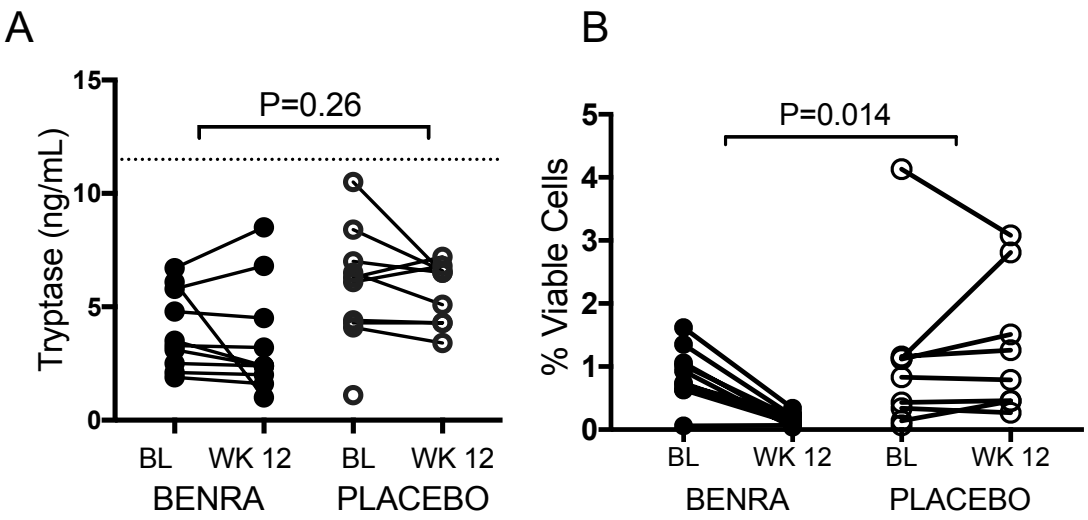


*Figure S2. Longitudinal suppression of AEC in response to benralizumab*



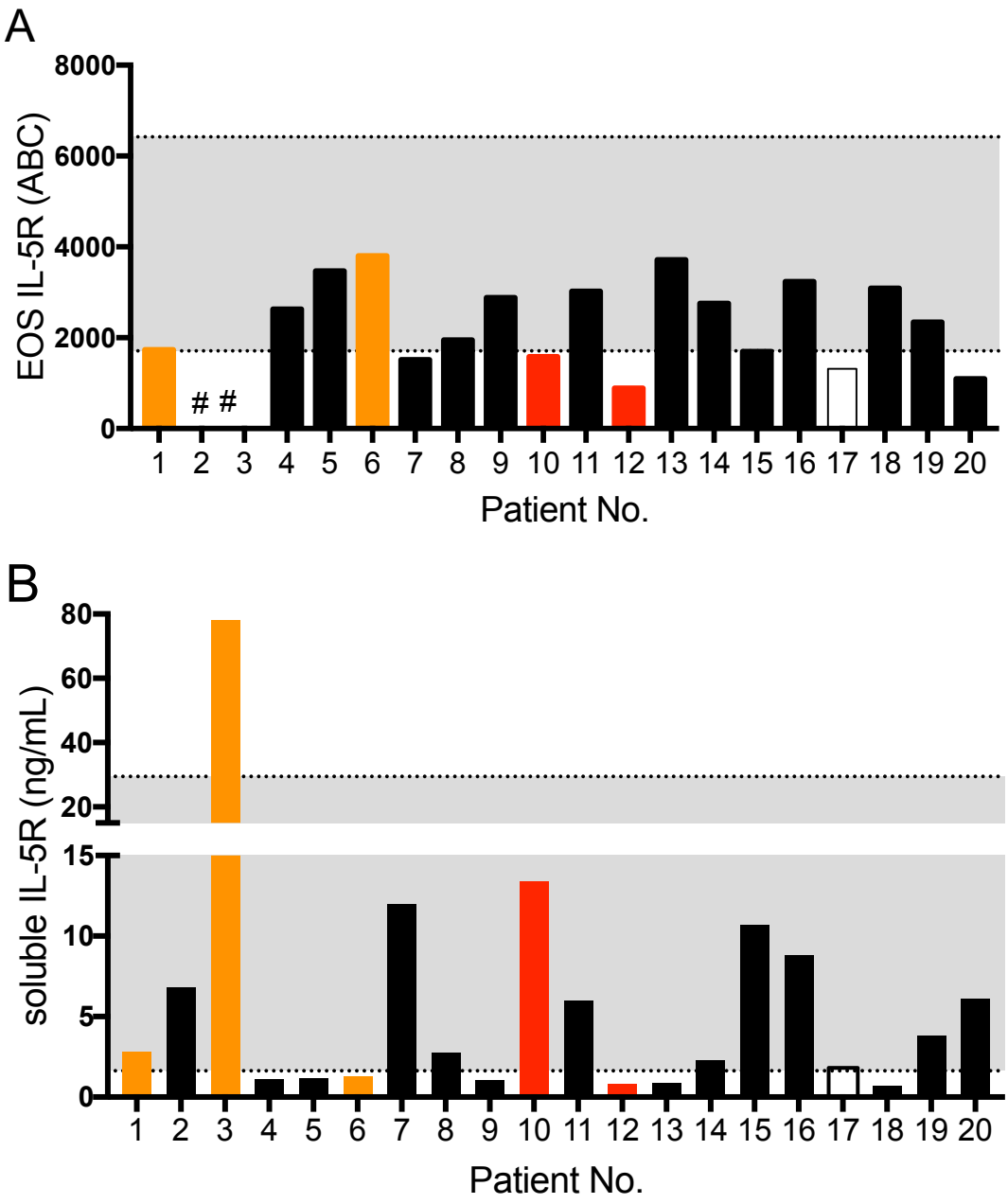

Plots demonstrate the AEC in cells/mm$^3$ over time for each individual patient between baseline and week 48. Responders are shown in black, non-responders in red and patients who relapsed in yellow. Patient 17 (placebo) withdrew from the trial prior to the open-label drug segment (green). The gray shaded area indicates the time period during which a patient received placebo. The horizontal dotted line indicates an AEC of 1,000 cells/mm$^3$.

*Figure S3. Effect of Benralizumab or placebo on serum tryptase levels and peripheral basophil counts at week 12*




A. Serum tryptase levels and B. peripheral blood basophils (CD123pos/HLA-DRneg) are shown at baseline and after 12 weeks of benralizumab or placebo therapy. Symbols represent the values for individual patients (closed = benralizumab group, open = placebo group). The horizontal dotted line indicates the upper limit of normal (12 ng/mL). Unadjusted P-value for two-sample t-test of differences is shown. With Holm's adjustment for multiple comparsions, $P_{adj}$=0.027 for peripheral blood basophils.

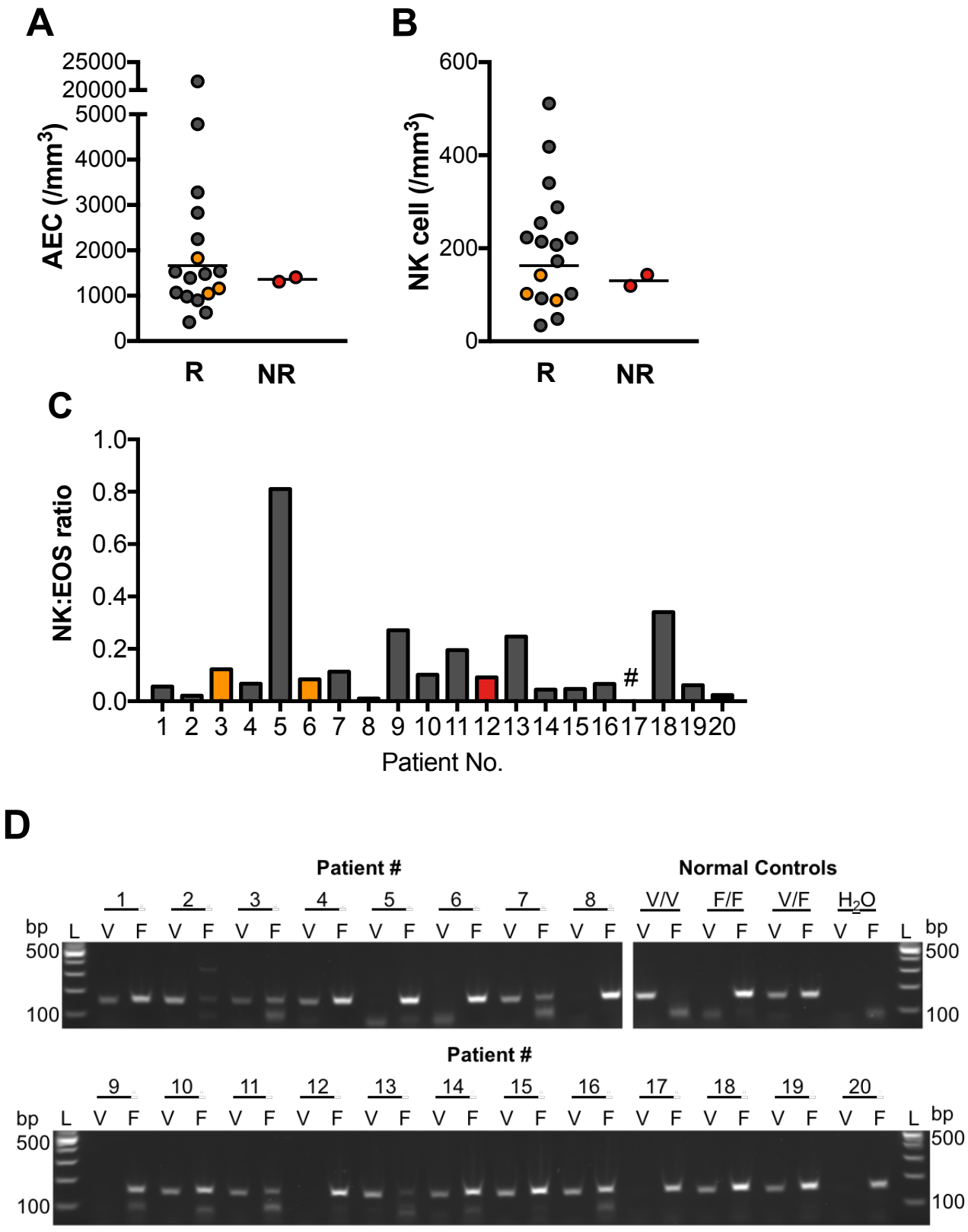
*Figure S4. Cell surface and soluble IL-5Rα levels at baseline*




A. Pre-treatment eosinophil cell surface IL-5Rα levels were assessed by whole blood flow cytometry and quantified. Antibodies bound per cell (ABC) are graphed. B. Pre-treatment plasma soluble IL-5Rα levels were assessed by sandwich ELISA. Pre-treatment is defined as Day 0 for patients randomized to benralizumab and Week 12 Day 0 for those randomized to placebo. Black bars indicate benralizumab responders,

orange bars indicate relapsers and red ones denotes non-responders. Open bar indicates patient (#17) who withdrew early. # indicates patients not assessed. Shaded areas demarcate 90% prediction intervals for healthy volunteers (n=31 for membrane IL-5R$\alpha$, n=16 for sIL-5R$\alpha$) using a normality assumption on log-transformed data.

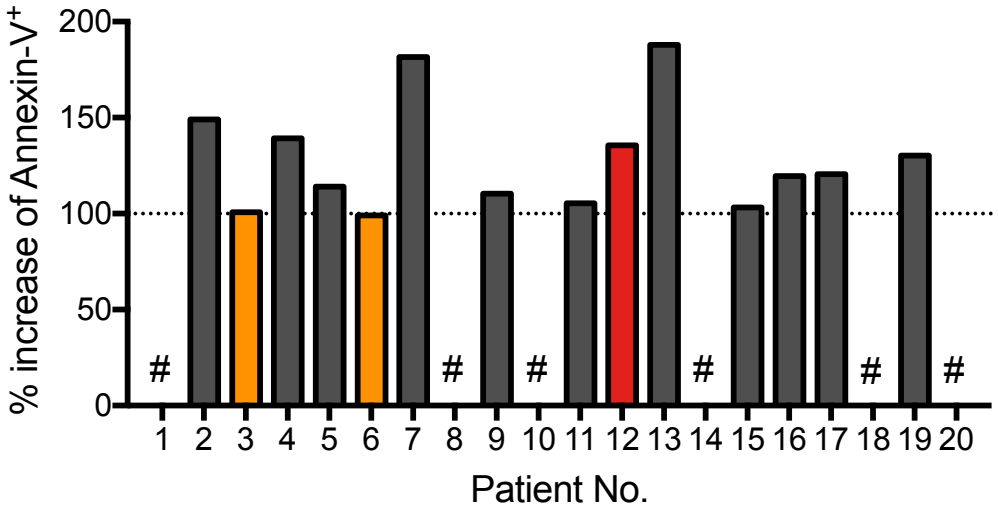
*Figure S5. NK cell parameters associated with response*



**E**

| Genotype | Patient # | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Homozygote (V/V) | 2 | 13 | | | | | | | | | |
| Heterozygote (V/F) | 1^ | 3^ | 4 | 7 | 10* | 11 | 14 | 15 | 16 | 18 | 19 |
| Homozygote (F/F) | 5 | 6^ | 8 | 9 | 12* | 17+ | 20 | | | | |

**F**

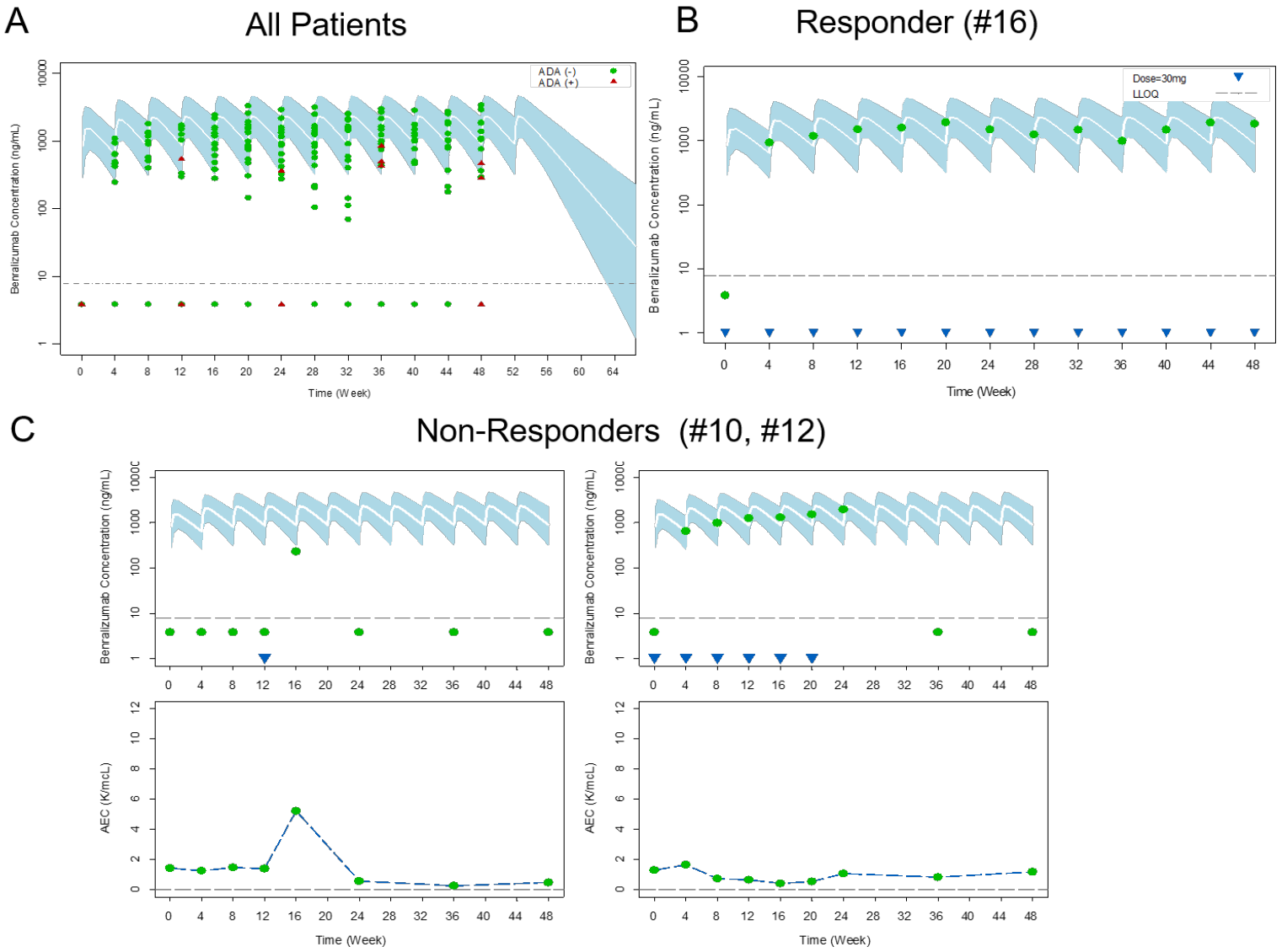



A-B) Graphs show the AEC and NK cell counts before the first dose of benralizumab. C) NK:EOS ratio plotted for each individual. Responders are indicated in black, non-responders in red and patients who relapsed in orange. # - indicates not done.

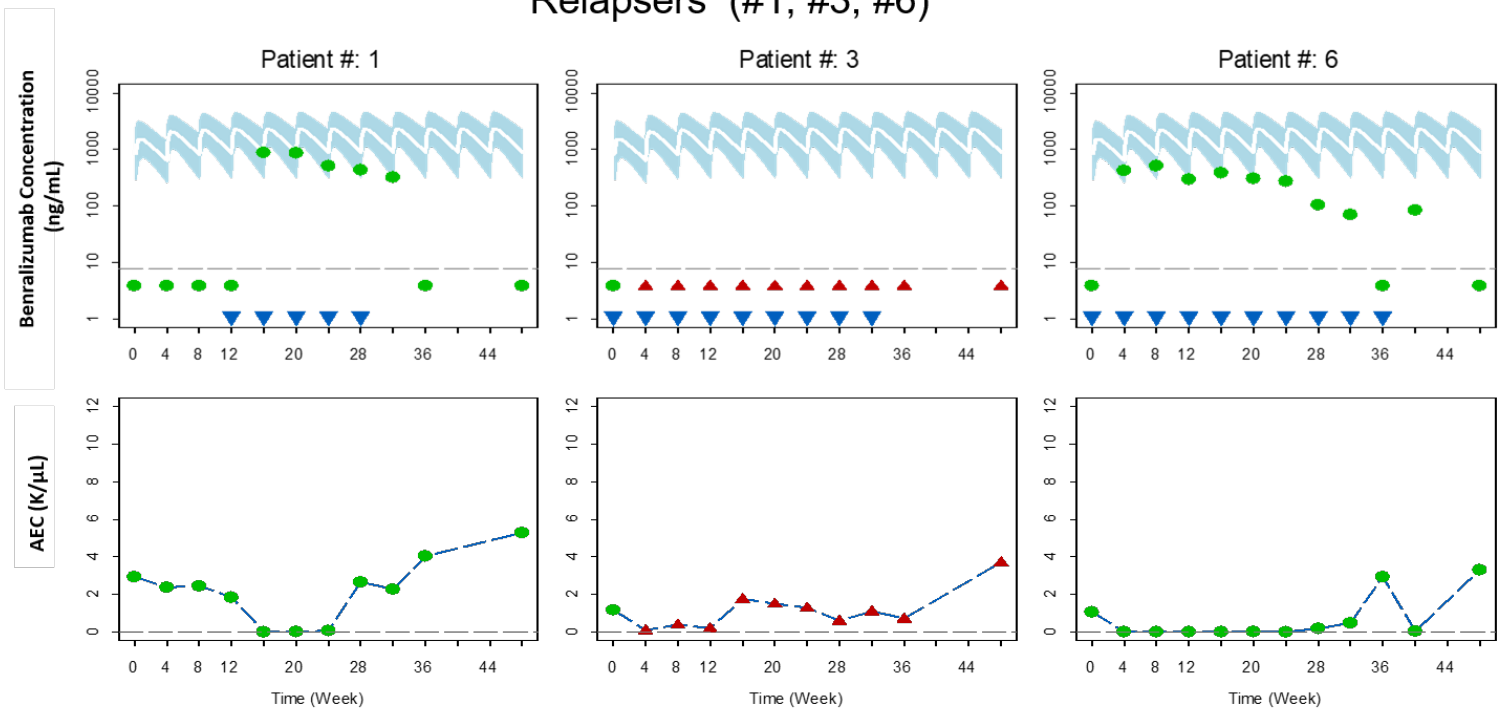
D) *CD16* polymorphisms - Each patient has a pair of lanes indicating allele-specific PCR products for the CD16 polymorphism. A positive band in the V lane indicates a valine at amino acid position 176, whereas a positive band in the F lane indicates a phenylalanine at position 176. Homozygosity for the F allele is associated with less effective CD16-mediated killing. Results from normal healthy controls are shown in the upper right gel. E) Table summarizing genotypes of benralizumab patients; *non-responder to benralizumab, ^ patient who relapsed while on benralizumab,

[+]patient who received placebo and withdrew at week 6 F) *In vitro* NK killing of eosinophils observed for each patient. Assays were performed on blood samples before the first administration of benralizumab (at the screening or baseline visit). Percent increase in annexin V-positive eosinophils over isotype control is shown.

*Figure S6. Population and Individual Benralizumab PK and AEC profiles in HES patients*



D




E

| ADA Positive | No. of Patients | Affected Patients |
|---|---|---|
| At any point during the trial | 5 /19 (26%) | #3, #7, #9, #15, #18 |
| Post-benralizumab only | 3 /19 (16%) | #3, #9, #15 |
| With effect on drug levels | 2 /19 (11%) | #3, #9 |

PK profiles for 5,000 virtual patients (with body weight and baseline eosinophil count random sampled from SIROCCO and CALIMA and the dosing schedule used in this HES trial) were simulated using PK model derived from the nine clinical

trials of benralizumab in asthma. Solid curve: median of simulated PK data; shaded area: 90$^{th}$ prediction interval (PI) delimited by the fifth and 95th percentiles of the simulated PK data. The horizontal dashed line represents the lower limit of quantitation (LLOQ). A) all patients, B) a representative responder, C) non-responders and D) relapsers. Observed drug concentrations are represented by a green circle if the patient did not have concurrent positive ADA, or a red triangle if they did. Blue triangles at the bottom of the graph denote each dose of 30 mg benralizumab. Concurrent AEC for graphs in B thru D are provided below each drug concentration graph. Note that non-responder #10 was placed on an alternative therapy beginning at week 20. E. Number of patients found to have ADA-positive status and specific patients affected for each category are listed.

**Clinical Vignettes**

**Patient 1** is a 53-year old woman who developed marked eosinophilia (>10,000 cells/$mm^3$), recurrent angioedema and pruritic skin rash at age 12. Evaluation was unrevealing and she was treated with intermittent topical and intramuscular glucocorticoids with transient relief. She was referred to the National Institutes of Health in December 2013 (at age 50) after a severe episode of angioedema requiring systemic glucocorticoid treatment. After treatment with prednisone 40 mg PO, her symptoms resolved and her AEC decreased to 1,500 cells/$mm^3$. At the time of initial NIH evaluation, she had discontinued prednisone therapy and was noted to have swelling of the hands restricting her ability to clench her fists, erythematous patches on her arms, palms, and lower legs. AEC was 12,480 cells/$mm^3$ on no therapy. A clonal population of T lymphocytes with an aberrant phenotype (CD3-CD4+) comprising 30% of all lymphoid cells was identified, consistent with lymphocytic variant HES. Extensive evaluation including CT scan of the chest, abdomen and pelvis and bone marrow biopsy revealed no evidence of malignancy. Shortly after her visit, she developed recurrent angioedema and was restarted on prednisone 40 mg. Repeated attempts to taper below 20 mg prednisone were unsuccessful with return of symptoms and AEC 3,100 cells/$mm^3$ after 2 weeks on prednisone 18 mg daily. She was enrolled on the benralizumab trial on 5/19/14 with persistent symptoms of fatigue and throat and extremity swelling. AEC was 2,930 cells/$mm^3$ on prednisone 18 mg daily. She received placebo for the first 3 months during which time she noted no improvement and had to increase her prednisone dose to 20 mg for increased throat swelling and discomfort. Following the first dose of open-label benralizumab, she reported decreased fatigue and improved hand swelling (she could remove her wedding ring for the first time in 4 months). AEC at week 13 was 60 cells/$mm^3$ (compared to 460 cells/$mm^3$ at week 12) and she was able to taper her prednisone dose to 5 mg daily with AEC 0 cells/$mm^3$ over the ensuing 18 weeks. At week 22, she developed worsening swelling with subcutaneous nodules and a cough. AEC was 70 cells/$mm^3$. She was treated with doxycycline for bronchitis. By week 24, her symptoms had significantly worsened and AEC was elevated at 2,540 cells/$mm^3$. Prednisone was increased to 12 mg with only transient improvement. Benralizumab was discontinued at week 32 for lack of efficacy. At week 48, AEC was 5,200 cells/$mm^3$ on interferon-$\alpha$ (1.75 mU SC daily) and prednisone (17 mg PO daily).

**Patient 2** is a 67-year old man with a history of mild eczema treated with topical corticosteroids who presented in August 2012 with fever, dyspnea, pruritic rash and diarrhea. AEC was 12,000 cells/$mm^3$. Gastrointestinal biopsies showed no evidence of eosinophilia and the skin findings were felt to be consistent with a drug reaction. Rosuvastatin was discontinued without effect. Extensive evaluation revealed a clonal T cell population in the peripheral blood and markedly elevated serum IgE. Moderate axillary lymphadenopathy in the axillary and inguinal areas was noted on CT scan of the chest, abdomen and pelvis. Positron emission tomography did not show increased metabolic activity; however, an axillary lymph node biopsy showed atypical lymphocytes that were CD30+ prompting concern for a lymphoid malignancy. He was started on prednisone 60 mg and received two cycles of brentuximab (anti-CD30 antibody) with only moderate control of his eosinophilia and no improvement in his symptoms. He was

subsequently treated with varying doses of prednisone, cyclosporine (150 mg PO BID), interferon-$\alpha$ (maximum dose 6 mU SC three times weekly), and hydroxyurea with minimal improvement and significant adverse effects. He was re-evaluated in 2013 off therapy. A second lymph node biopsy showed only dermatopathic changes without evidence of lymphoma. Skin biopsy showed non-specific findings with eosinophilia. AEC at the time was 21,700 cells/mm3. He was given a diagnosis of HES and treated with imatinib without response. Upon presentation to the NIH, he complained of fatigue, intermittent low-grade fever, itching, skin redness, especially on the arms and legs, dyspnea with exertion, occasional wheezing, and headaches. Physical exam was notable for erythema of skin primarily the face, arms, and necks, with thickening and scaling on the lower extremities. AEC was 6,010 cells/$mm^3$ on prednisone 20 mg daily. IgE was elevated at 1,534 IU/mL. T cell receptor rearrangement showed a clonal pattern in the blood. No aberrant phenotype was detectable by flow cytometry. CT scan of the chest, abdomen and pelvis showed no evidence of malignancy. Multiple skin biopsies showed atypical lymphocytic infiltrates with loosely formed granulomas and no evidence of clonality by PCR. At the time of enrollment, AEC was 9,800 cells/$mm^3$ on prednisone 20 mg PO daily. He was randomized to receive benralizumab. Approximately 2 hours after the injection, he developed a headache, but felt well enough to go back to the hotel. By early evening, he developed severe fatigue, subjective fever, chills, nausea and loss of appetite, but did not seek medical attention. By the time he was seen in clinic the next morning, the symptoms had resolved. LDH was elevated at 408 U/L and urinalysis was notable for new 1+ proteinuria. Both had resolved by the following day. AEC at week 13 was 0 cells/$mm^3$ and a prednisone taper was initiated. His course was complicated by 1) hospitalization at week 15 for a renal stone of unknown composition requiring cystoscopic removal and temporary stent placement and 2) overnight hospital admission at week 21 for presumed infectious colitis treated with rehydration and a single dose of levofloxacin and metronidazole. Despite an AEC of 0 cells/$mm^3$, attempts to taper prednisone below 10 mg daily were unsuccessful due to recurrent fatigue and nausea, initially thought to be related to adrenal insufficiency. A cosyntropin stimulation test was normal. Physical examination was notable for erythematous macular skin lesions different in character from his prior dermatitis. At week 36, he presented with hypercalcemia (calcium 3.08 mmol/L), which was treated acutely with hydration and calcitonin. Serum creatinine was elevated above baseline. Skin biopsy was performed and showed lymphocytic infiltration with non-necrotizing granulomas. Given these findings and review of the prior bone marrow biopsies, which also showed evidence of loosely formed granulomas, the overall clinical picture was felt to be consistent with sarcoidosis, likely unmasked by the steroid taper. His prednisone dose was increased to 40 mg daily with improvement. At week 48, AEC was 0 cells/$mm^3$ on prednisone 40 mg daily.

**Patient 3** is a 46-year old woman with a history of severe pruritus and angioedema resistant to antihistamines since January 2008. She was diagnosed with HES and idiopathic thrombocytopenic purpura (ITP) in January 2009, when she was noted to have an AEC of 13,000 cells/$mm^3$ and platelets of 34,000. She was treated with prednisone 60 mg daily with good response. Multiple attempts to lower the prednisone

dose were unsuccessful, and her AEC rose to 26,000 cells/mm$^3$ by September 2009. Hydroxyurea was started as a steroid-sparing agent, with moderate success. AEC was 3080 cells/mm$^3$ and symptoms were improved on prednisone 10 mg daily and hydroxyurea 500 mg alternating with 1,000 mg daily. Extensive evaluation revealed CD3-CD4+ lymphocytic variant HES without evidence of lymphoma. Interferon-$\alpha$ therapy was initiated. Over the next 5 years, she continued to have fatigue, intermittent angioedema and pruritus resulting in widespread excoriations and scarring. She was unable to taper the prednisone and had an AEC of 1,160 cells/mm$^3$ on interferon-$\alpha$ 1 mU SC daily and prednisone 12.5 mg PO daily at the time of enrollment. She received her first dose of benralizumab on day 0 complicated by headache, fever, chills, and nausea that resolved by the following day. The next morning, LDH was elevated at 203 U/L. At week 13, her AEC was 0 cells/mm$^3$ and interferon-$\alpha$ was discontinued. At week 14, her AEC had risen to 150 cells/mm$^3$, but she reported improved symptoms. By week 16, her symptoms had increased significantly with recurrence of pruritus and swelling. AEC was 1,750/mm$^3$. She received a second dose of open-label benralizumab without immediate effect. Interferon-$\alpha$ was restarted at 1 mU SC daily with improvement in her symptoms, including resolution of swelling. Between weeks 20 and 32, her symptoms remained relatively well-controlled with AEC fluctuating between 370 and 1,270 cells/mm$^3$ on the same doses of interferon-$\alpha$ and prednisone that she had been receiving prior to the trial. At week 36, however, she reported increased swelling and pruritus prompting discontinuation of benralizumab therapy. At week 48, AEC was 3,700/mm$^3$ on interferon-$\alpha$ 1 mU and prednisone 12.5 mg daily.

**Patient 4** is a 37-year old woman with a history of lymphocytic variant HES manifested as severe spongiotic dermatitis since 2009, complicated by superinfections and bacteremia resulting in multiple hospitalizations. Previous therapies included prednisone (40 mg PO daily), phototherapy, methotrexate, cyclosporine, mycophenolate, thalidomide, interferon-$\alpha$, and various topical agents. None of these therapies adequately controlled her skin disease or eosinophilia, which ranged from 1,870-9,570 cells/mm$^3$. Pruritic, painful, skin lesions flared periodically and were disruptive to the point that she required narcotics to control the pain, was unable to work and rarely left her home, becoming socially isolated. She kept as much of her body covered as possible, even wearing gloves during the summer, and her clothes often became damp due to weeping of vesicular skin lesions. At baseline, her AEC was 1,650 cells/mm$^3$ on pegylated interferon-$\alpha$ (90 mcg SC weekly). Her physical examination was notable for diffuse dermatitis (see Figure 2), lymphadenopathy and alopecia. She received placebo during the initial segment, then received her first dose of benralizumab at week 12. At week 13, AEC was 30/mm$^3$ and declined further to 0 cells/mm$^3$ at week 15. Although her skin lesions were unchanged, she noted a decrease in lymphadenopathy. Pegylated interferon-$\alpha$ was discontinued, and wet wrap therapy was initiated for 4 days with some improvement in her dermatitis. Over the next 6 months, her skin symptoms continued to improve. She had no skin infections or hospitalizations and was able to discontinue narcotics. Moreover, she was no longer depressed, had gained weight, and felt comfortable leaving the house in weather-appropriate clothing. At week 48, AEC was 0 cells/mm$^3$ on benralizumab monotherapy with occasional mild flares of her skin lesions.

**Patient 5** is a 58-year old man with idiopathic HES who developed fatigue and dyspnea on exertion in his mid-40s requiring multiple hospitalizations and at least one intubation. Significant peripheral eosinophilia was noted in 2011, and bone marrow evaluation showed increased eosinophils without other abnormality. He subsequently developed soft tissue swelling in his neck consistent with angioedema. Prior treatments include high-dose prednisone (25-60 mg daily), cyclosporine, and pegylated interferon-$\alpha$. His symptoms at baseline included chest tightness, dyspnea, and soft tissue swelling of the hands, feet, neck and chest. Physical exam was notable for a cushingoid appearance and lower extremity edema. AEC was 3,940 cells/mm$^3$ on pegylated interferon-$\alpha$ (50 mcg weekly) and prednisone (17.5 mg daily). He was randomized to placebo and received his first dose of active drug at week 12 without incident. His AEC was 0 cells/mm$^3$ at week 13 with improvement in his symptoms. Interferon-$\alpha$ was discontinued. His course was complicated by an episode of hypomanic symptoms beginning at week 14, that resolved with discontinuation of fluoxetine (which had been initiated for interferon-$\alpha$-related depressive symptoms) and tapering of prednisone therapy. At week 20, shortly after reducing his prednisone dose to 10 mg PO daily, he presented with localized urticarial lesions on his back and abdomen. Of note, he had a distant history of chronic spontaneous urticaria that had resolved on prednisone therapy. At Week 48, AEC was 0 cells/mm$^3$ and he remained asymptomatic on benralizumab monotherapy with the exception of intermittent urticaria.

**Patient 6** is a 48-year old woman with a history of marked eosinophilia, abdominal pain and diarrhea since 2009. Lymphocytic variant HES was diagnosed in 2012 based on clonal T cell receptor studies and an aberrant CD3-CD4+ T cell population on flow cytometry. Prior therapies, including prednisone, hydroxyurea, and interferon-$\alpha$ were ineffective or had intolerable side effects. At baseline, AEC was 2,480 cells/mm$^3$ and her symptoms included fatigue limiting her activities of daily living, neuropathy, abdominal pain and diarrhea, despite prednisone 10mg and hydroxyurea 1000mg daily. Endoscopy and colonoscopy revealed eosinophilic gastritis. She received her first dose of benralizumab on day 0 complicated by headache that resolved by the next day. On the following day, LDH was elevated at 233 U/L. Hydroxyurea was discontinued at week 13 and prednisone was slowly tapered. Her diarrheal symptoms resolved by week 16, and she reported less fatigue. Repeat endoscopy and colonoscopy were performed at week 24. Biopsies of all segments were notable for the complete absence of eosinophils. At week 28, her AEC rose to 170 cells/mm$^3$ on prednisone 5 mg daily, and her diarrhea began to return. At week 36, her AEC was 2,920/mm$^3$ and her symptoms had returned to baseline levels, including diarrhea, fatigue, and abdominal pain severe enough to require narcotic pain medications. Her prednisone dose was increased to 20 mg daily with some improvement in her symptoms and resolution of the eosinophilia. However, reduction of the prednisone dose to 15 mg daily resulted in an increase in the severity of her symptoms. Benralizumab was discontinued at week 40. At week 48, the severity of her symptoms was comparable to that prior to enrollment on the trial and AEC was 3,300 cells/mm$^3$ on prednisone 15 mg daily.

**Patient 7** is a 38-year old man with a history of marked eosinophilia (peak AEC 4,400 cells/mm$^3$) and gastrointestinal symptoms, including abdominal pain, diarrhea, weight

loss, dysphagia, and odynophagia exacerbated by eating. Prior therapies, including prednisone, food elimination, budesonide, hydroxychloroquine, sirolimus, and swallowed fluticasone, were ineffective. A practicing physician, he managed his symptoms at work by fasting. Baseline EGD revealed >50 eosinophils/hpf in his proximal and distal esophagus and >100 eosinophils/hpf in the stomach despite budesonide 9 mg daily and swallowed fluticasone. AEC was 2,250 cells/$mm^3$. He received his first dose of benralizumab on day 0 complicated by headache, fever, and chills that resolved within 24 hours. On the following day LDH was elevated at 250 U/L. Repeat EGD at week 24 showed no eosinophils in the esophagus, stomach, duodenum or colon. At week 48, his AEC remained 0 cells/$mm^3$ and he was able to eat meals during work hours despite tapering the dose of budesonide to 6 mg daily. He continued to have occasional flares of abdominal pain that resolved without intervention.

**Patient 8** is a 57-year old woman with a history of urticaria, sinusitis with nasal polyps, and marked eosinophilia (AEC ranging from 7,726 cells/$mm^3$ in 2005 to 23,400 cells/$mm^3$ in 2011). In January 2013, she presented with dyspnea and palpitations and was found to have severe mitral regurgitation, marked bilateral atrial enlargement, and severe pulmonary hypertension, prompting mitral valve replacement and tricuspid valve repair. She was started on prednisone, but this failed to control her eosinophilia (AEC 33,700 cells/$mm^3$), and she developed severe stenosis of her bovine mitral valve. She was treated with high dose steroids, hydroxyurea, and ultimately a single dose of cyclophosphamide (750 mg/$m^2$) prior to mitral valve annuloplasty less than 4 months after her initial cardiac surgery. Pathology of the excised valve tissue showed eosinophilic infiltration. Following the second surgery, she was treated unsuccessfully with interferon-$\alpha$ (1 mU sc daily) and mepolizumab (700 mg intravenously every 4 weeks). She resumed monthly cyclophosphamide with only partial suppression of her hypereosinophilia and debilitating side effects including fatigue, malaise, nausea, and vomiting lasting for 3-5 days after each dose. At the time of enrollment on the benralizumab trial, her AEC was 7,250 cells/$mm^3$. She received placebo during the first 3 months during which time she reported intermittent skin rash and increasing dyspnea on exertion for which she saw her local cardiologist. Echocardiography was unchanged. At week 12 she received her first dose of benralizumab without incident. AEC at the time was 3,280 cells/$mm^3$. By week 13, her AEC had decreased to 20 cells/$mm^3$ and cyclophosphamide was discontinued. Her quality of life improved immensely, although she continued to have intermittent urticaria responsive to antihistamines and experienced self-limited grade 1 local injection site reactions on multiple occasions following both placebo and benralizumab. AEC at week 48 was 0 cells/$mm^3$, and echocardiography showed no change from prior studies.

**Patient 9** is a 31-year old woman with a history of asthma, sinus disease with polyps S/P multiple surgical procedures, recurrent eosinophilic pulmonary infiltrates, localized angioedema and urticaria beginning in 2007. An elevated AEC (3,300 cells/$mm^3$) was first documented in 2010. Subsequent bronchoscopic and sinus biopsies performed on no systemic therapy showed tissue eosinophilia, but no evidence of vasculitis. Serum anti-neutrophil cytoplasmic antibodies were not detected. She was treated with high doses of prednisone with good response, but was unable to taper below prednisone 20

mg daily due to recurrent symptoms and eosinophilia. Interferon-α 1 mU SC daily was initiated in October 2012, again with good response, but was discontinued in February 2014 due to insurance issues. At the baseline visit on the benralizumab trial, AEC was 1,760 cells/mm$^3$ on prednisone 25mg daily and 2 maintenance inhalers (budesonide/formoterol 160mcg/4.5mcg 2 inhalations BID, fluticasone 220 mcg 1 inhalation BID) with audible wheezing diffusely on exam. She received placebo during the placebo-controlled segment. At week 6, formoterol 12.5 mcg inhaled BID was added to her regimen due to persistent wheezing and dyspnea. At week 12, she received her first dose of open-label benralizumab. She reported a mild headache and chills approximately 6 hours after the injection, which resolved within 30 minutes. LDH was slightly increased the next day from 202 to 216 U/L. By week 13, AEC was 0 cells/mm$^3$ and she noted profound improvement in her breathing. Formoterol was stopped and the prednisone dose was gradually tapered. At week 28, she complained of increasing asthma and sinus symptoms on prednisone 8 mg daily. Although her AEC remained suppressed at 0 cells/mm$^3$, the symptoms persisted and sinus CT scan showed complete opacification of the paranasal sinuses. She was treated with a short burst of prednisone with improvement. At week 36, she continued to be symptomatic, albeit improved. AEC was 40 cells/mm$^3$. Prednisone dose was decreased to 5 mg daily. At week 46, she underwent sinus surgery with placement of glucocorticoid-eluting stents. At week 48, she was asymptomatic on prednisone 5 mg daily with an AEC of 290/mm$^3$.

**Patient 10** is a 52-year old woman with a history of myeloid variant HES with a V617F mutation in *JAK2* complicated by erythrocytosis, cardiac and neurologic manifestations. She was initially diagnosed in 2004 when erythrocytosis and eosinophilia (1,250 cells/mm$^3$) were noted in the setting of severe bleeding after a miscarriage. She was diagnosed with presumptive polycythemia vera and treated with phlebotomy. Her AEC slowly increased from 1,250 to 4,067 cells/mm$^3$ by late July 2012. On August 2, 2012, a CT scan performed for evaluation of an enlarged lymph node revealed a new filling defect in the apex of the left ventricle of the heart. Cardiac MRI confirmed endomyocardial fibrosis with thrombosis and she was started on enoxaparin, prednisone 60 mg and imatinib 100 mg. Her AEC remained elevated and a trial of pegylated interferon-α was initiated. Although interferon-α showed efficacy in lowering her AEC, she developed severe thrombocytopenia (platelet count of 5,000/mm$^3$) necessitating platelet transfusion, intravenous immunoglobulin and discontinuation of interferon-α therapy. Her AEC began to rise and hydroxyurea was started in August 2014 at 500 mg daily. This resulted in recurrent thrombocytopenia prompting a decrease in dose to 500 mg three times weekly. In February 2015, she experienced abrupt onset of aphasia that lasted several minutes. MRI showed an acute infarct in the left frontal lobe and an old infarct in the right cerebellar hemisphere. Given the life-threatening manifestations and failure of multiple therapies, she qualified for and received 2 monthly doses of compassionate use mepolizumab (750 mg IV) without effect. Ruxolitinib therapy was considered but declined by the patient because of the fear of recurrent thrombocytopenia, and she instead enrolled on the benralizumab trial. At baseline, she complained only of fatigue with AEC 1,440 cells/mm$^3$ on hydroxyurea 500mg three times weekly. Randomized to placebo, she received her first dose of benralizumab at week 12 without adverse events. Although her AEC declined to 850

cells/mm$^3$ on the next day, it had rebounded to baseline levels by week 13 and increased to 5,220 cells/mm$^3$ by week 16. Benralizumab therapy was discontinued because of lack of response and ruxolitinib therapy was initiated. At week 48, she was doing well with resolution of fatigue and AEC 490 cells/mm$^3$ on ruxolitinib (20 mg twice daily).

**Patient 11** is a 55-year old man with a history of marked eosinophilia (peak AEC 17,200 cells/mm$^3$), asthma, chronic sinusitis and gastrointestinal symptoms, including reflux, dysphagia, abdominal pain, abdominal bloating, anorexia, diarrhea, early satiety, and weight loss beginning in August 2013 when he presented with shortness of breath and abdominal symptoms. Laboratory evaluation was notable for AEC 6,040 cells/mm$^3$ and evidence of pancreatitis. Endoscopy and colonoscopy showed 20-30 eosinophil/hpf in the duodenum and >50 eosinophils/hpf in the stomach. Extensive evaluation, including assessment of anti-nuclear cytoplasmic antibodies and bone marrow examination, was otherwise unrevealing. He was treated with several courses of oral prednisone, as well as crushed budesonide, with partial response. At the time of enrollment, he had moderate symptoms, including fatigue, arthralgia, abdominal cramping and loose stools on budesonide 3 mg daily with AEC 1,480 cells/mm$^3$. Endoscopy again demonstrated eosinophilic gastritis and duodenitis, as well as eosinophilic esophagitis and colitis. He was randomized to drug and received his first dose of benralizumab on day 0. Several hours after receiving drug, he complained of transient flu-like symptoms including diffuse myalgias, flushing, and sweating. LDH on the following day was elevated at 248 U/L. Beginning at week 2, he reported clinical improvement. AEC at week 13 was 0 cells/mm$^3$ and repeat EGD and colonoscopy showed only a rare eosinophil in the duodenum and distal esophagus. Budesonide was discontinued at week 24 (3/10/16). Just prior to his week 32 visit, he was bitten by a tick and treated empirically for Lyme disease. He subsequently began to complain of mild fatigue, joint pain and dysphagia. Rheumatology evaluation was unrevealing and his symptoms were attributed to osteoarthritis exacerbated by the discontinuation of budesonide. Repeat endoscopy showed a ringed esophagus and furrows, but no evidence of eosinophils on biopsy. At week 48, his complaints were stable on no additional HES therapy with AEC of 0 cells/mm$^3$.

**Patient 12** is a 61-year old woman with myeloid variant HES with a *JAK2* mutation in exon 13. She was in good health until 2007 when she presented with asymptomatic erythrocytosis and marked eosinophilia. Testing for *JAK2* V617F was negative. She was treated with phlebotomy for presumed polycythemia vera for one year. Repeat bone marrow biopsy in 2011 was felt to be more consistent with an eosinophilic myeloid neoplasm. Fluorescence in situ hybridization for *FIP1L1-PDGFRA* was negative and cytogenetics were normal. After failing treatment with imatinib (400 mg daily), high dose prednisone and pegylated interferon-$\alpha$, she was followed on no therapy with AEC ranging from 14,300 to 19,600 cells/mm$^3$ until late July 2014, when she developed intermittent sensory and vision changes. AEC was 33,000 cells/mm$^3$. MRI showed acute and subacute infarcts. Genetic testing at this time revealed a deletion in *JAK2* exon 13. She was treated with a single dose of IV cytarabine and started on hydroxyurea (1 g twice daily) with partial reduction of her AEC to 10,300 cells/mm$^3$.

Hydroxyurea was discontinued and alemtuzumab (anti-CD52) therapy was initiated in August 2014 with resolution of eosinophilia (AEC 200 cells/$mm^3$) but development of lymphopenia and CMV reactivation after less than 1 month of therapy. Alemtuzumab was restarted in November 2014 with CMV reactivation after only 9 days of therapy. She remained off therapy until January 2015 when hydroxyurea (1 g twice daily) was restarted because of a rising AEC (8,300/$mm^3$). Although this reduced her eosinophil count, she became profoundly thrombocytopenic and hydroxyurea was discontinued in February 2015. Hydroxyurea was ultimately restarted at a lower dose (500 mg five times per week) and she was enrolled on the benralizumab study in November 2015. At baseline evaluation, she complained of fatigue, weakness, dyspnea and residua of her prior strokes. AEC was 1,310 cells/$mm^3$. She received her first dose of benralizumab on day 0. Her AEC ranged from 570 to 1,660 cells/$mm^3$ during the first twelve weeks of the trial. Although her AEC at week 13 (1,170 cells/$mm^3$) was not significantly changed from baseline and her symptoms remained unchanged, she received benralizumab monthly until week 24, at which time it became clear that she was not responding. At week 48, she remained symptomatic on hydroxyurea monotherapy with an AEC of 1,080 cells/$mm^3$.

**Patient 13** is a 37-year old woman with a history of marked eosinophilia, fatigue and watery diarrhea (up to 20 episodes daily) since 2008. She attempted dietary restriction without relief and did not seek medical attention until 2014 at which time she underwent colonoscopy which showed 50-100 eosinophils/hpf. She was started on budesonide 9 mg daily with improvement in her symptoms, but significant adverse effects. Tapering the budesonide dose to 6 mg daily caused return of her symptoms. Patch testing for a wide variety of foods was negative. She tried an elemental diet, which decreased the frequency of her diarrhea, but was unsustainable. Cromolyn was only transiently effective. Over the next year, she developed pruritic skin lesions, night sweats and bone pain. Peak AEC during this time was 2,602 cells/$mm^3$. Extensive evaluation, including bone marrow examination, was unrevealing. Although oral prednisone therapy (10 mg daily) reduced her symptoms, she experienced severe headaches and weight gain. At the time of enrollment, she complained of watery diarrhea 5-7 times daily despite loperamide, nausea, night sweats, joint pain, nasal congestion and intermittent pruritic rash. These symptoms impacted her daily life and prevented her from working in her previous occupation as an exercise instructor. AEC was 1,000 cells/$mm^3$. Baseline endoscopy and colonoscopy was performed and revealed 50-150 eosinophils/hpf in the colon and 10-25 eosinophils/hpf in the duodenum. She was randomized to placebo. She received her first dose of benralizumab at week 12 without adverse events. At week 13, her AEC was 0 cells/$mm^3$ and she reported symptomatic improvement. Repeat endoscopy and colonoscopy at week 24 were notable for the absence of pathologic changes and eosinophilia in all segments biopsied. At week 48, she continued to do well with markedly improved symptoms despite a liberalized diet and AEC of 0 cells/$mm^3$.

**Patient 14** is a 23-year old woman with a long history of eosinophilic gastritis, eosinophilic esophagitis, IgE-mediated food allergies to milk and wheat, exercise-induced asthma, and peripheral eosinophilia. She presented in 2003 at 10 years of age

with severe anemia that did not respond to iron repletion and was found to have eosinophilic gastritis and esophagitis with an AEC of 2,930 cells/$mm^3$. Over time, she developed increasing gastrointestinal symptoms, including abdominal pain, nausea, dysphagia, and diarrhea, as well as exercise-induced asthma, daily headaches, joint pains, fatigue, night sweats and mild rhinitis. Beginning in 2003, she was treated with an empiric six-food elimination diet, elemental formula, swallowed fluticasone, crushed budesonide, cromolyn, proton pump inhibitors, anti-histamines, sirolimus and azathioprine with variable efficacy and significant side effects. Her AEC varied between 1,000 and 4,300 cells/$mm^3$ during this time. At baseline, her AEC was 1,070 /$mm^3$ on swallowed fluticasone twice daily. Her diet was restricted to Neocate (8 oz daily) and eight foods (shrimp, pear, apple, banana, lemon, potato, broccoli and lettuce). Endoscopic evaluation confirmed continued tissue eosinophilia with >100 eos/hpf in both the esophagus and stomach, and >30 eos/hpf in the duodenum. She received benralizumab on day 0 without adverse events. Her AEC was 0 cells/$mm^3$ by week 2. Repeat endoscopic evaluations at week 24 demonstrated no eosinophils in the esophagus, stomach, duodenum or colon. Over the remaining 24 weeks, her AEC remained 0 cells/$mm^3$ and she began to reintroduce new foods into her diet. At week 48, her AEC remained suppressed at 0 cells/$mm^3$ and she was able to add fish, poultry, tomato and carrots to her diet, although she no longer tolerated shrimp.

**Patient 15** is a 34-year old woman with lymphocytic variant HES who presented in 2002 with a 30-pound weight gain and urticaria. AEC was 13,400 cells/$mm^3$. In 2007, she developed severe watery diarrhea and dysphagia. Colonoscopy revealed eosinophilic colitis. Finally, in 2009, her symptoms progressed to include joint pains and fatigue. Her AEC during this time was persistently elevated between 4,400 and 10,000 cells/$mm^3$ and she was started on prednisone 80 mg daily with symptomatic improvement. Her AEC decreased to 500-700 cells/$mm^3$ on prednisone 80 mg daily but rebounded each time she tapered below 60 mg daily. Evaluation in 2010 was notable for an aberrant CD3-CD4+ T cell population. Interferon-$\alpha$ (1 mU SC daily) was started in July 2011. Although interferon-$\alpha$ allowed her to reduce the prednisone dose to 5 mg daily, it had to be discontinued in October 2011 because of severe depression. In March 2012, she developed cholecystitis while on prednisone 20 mg daily. The pathology showed acute cholecystitis with eosinophilic infiltration. Prednisone was stopped at this time because of lack of efficacy and significant toxicity. She remained off HES therapy despite symptoms and markedly elevated AEC until enrollment in the benralizumab trial. At baseline, she reported pruritus, fatigue, intermittent dysphagia, and severe diarrhea immediately after eating, sometimes lasting all day or all week. Her AEC was 5,620 cells/$mm^3$. Baseline endoscopic evaluation was notable for eosinophils (up to 47 eosinophils/hpf) throughout the lower GI tract. She was randomized to placebo and received her first benralizumab dose at week 12 followed by chills and a headache three hours later which resolved by the evening. The following day LDH rose to 299 U/L. At week 13, her AEC was 0 cells/$mm^3$ and she noted complete resolution of her symptoms, including fatigue, joint pain and diarrhea. She reported formed stools for the first time in many years and was able to eat normally. Follow-up endoscopy and colonoscopy at week 24 was notable for the absence of eosinophils in all segments.

With the exception of an acute and self-limited gastrointestinal illness at week 45, she remained asymptomatic for the remainder of the trial with AEC of 0 cells/$mm^3$.

**Patient 16** is a 35-year old woman who presented at age 7 with peripheral eosinophilia and abdominal pain, reflux, nausea, and vomiting that led to frequent school absences. Asthma was diagnosed at age 19, and biopsies confirmed eosinophilic infiltration of the esophagus and stomach at age 22. Since that time, she has developed dysphagia (requiring at least one esophageal dilation), food sticking, bloating, constipation, occasional diarrhea, iron-deficiency anemia and multiple food sensitivities, including IgE-mediated food allergies to egg and shrimp. AEC ranged from 1,200-2,000 cells/$mm^3$. Beginning in 2004, she was treated with prednisone, interferon-$\alpha$, budesonide, swallowed fluticasone, cromolyn, and sirolimus with limited improvement. At baseline, her AEC was 1,390 cells/$mm^3$ on swallowed fluticasone with limited intake of the following foods: rice, pure egg, shrimp, shellfish, corn, bread, meats, and raw vegetables. Baseline endoscopy demonstrated >100 eosinophils/hpf in the distal esophagus and stomach. She received her first dose of benralizumab on day 0 without adverse events. AEC was 100 cells/$mm^3$ by the next day and dropped to 0 cells/$mm^3$ by week 2. At week 18, she began to notice decreased nausea. Her course was complicated by hospitalization and passage of a calcium oxalate kidney stone at week 23. Follow-up endoscopy at week 24 demonstrated no eosinophils in any GI segment. At week 48, she continued to have intermittent gastrointestinal symptoms, albeit milder and less frequent than prior to starting benralizumab therapy. AEC remained suppressed at 0 cells/$mm^3$.

**Patient 17** is a 74 year-old man who presented with peripheral eosinophilia, palmar erythema, swelling and generalized fatigue of 16 years duration. On initial evaluation, he was found to have a palpable spleen tip and bone marrow eosinophilia, but no evidence of a myeloid or lymphoid malignancy. He was treated sequentially with prednisone, imatinib (400 mg PO twice daily), hydroxyurea (1500 mg twice daily), and interferon-alpha (up to 4 million units daily) without improvement. Mepolizumab 750mg IV monthly was also ineffective in reducing his eosinophilia or controlling his symptoms. Additional agents tried, but ineffective, included methotrexate, cyclophosphamide, dasatinib, mycophenolate mofetil, pegylated interferon-alpha with hydroxyurea, alemtuzumab (30 mg SC three times weekly for two months) and cladrabine. During the past 9 years, he was treated with prednisone ranging from 10-50mg daily with or without hydroxyurea. Side effects related to chronic prednisone included worsening diabetes, glaucoma, Cushingoid body habitus, proximal myopathy and muscle wasting, and osteoporosis. He was diagnosed with hypothyroidism likely secondary to interferon-alpha. In recent years, he experienced frequent pulmonary and urinary infections, several resulting in hospitalization, as well as frequent falls resulting in shoulder injuries and deconditioning. He was enrolled on the benralizumab trial on prednisone 30 mg and hydroxyurea 500 mg daily with an AEC 2,370 cells/$mm^3$. His HES symptoms at baseline included myalgia, arthralgia, and fatigue. He was also noted to have chronic bilateral pulmonary ground-glass opacities on chest CT. He was randomized to placebo and, at his week 6 visit, was hospitalized at NIH for hypotension and confusion, which was ultimately determined to be due to over-medication of his blood pressure in the setting

of volume depletion. He withdrew from the trial after the second dose of study drug (placebo) when his wife became ill and could no longer accompany him to study visits.

**Patient 18** is a 31-year old man who presented in January 2015 with headaches and myalgias after a trip to India and was found to have an AEC of 26,950 cells/mm$^3$. Evaluation revealed no evidence of a secondary cause. He subsequently developed a non-productive cough, diarrhea, fever, and hypoxia. Chest CT showed pulmonary infiltrates and bronchoalveolar lavage was notable for a white blood cell count of 10,054 with 72% eosinophils. He was admitted to the ICU and treated with IV methylprednisolone and imatinib without response (AEC 47,120 cells/mm$^3$). His course was complicated by the development of a splenic infarct and multiple extremity deep venous thromboses. Peak AEC during the hospitalization was 86,180 cells/mm$^3$. He was discharged on hydroxyurea, rivaroxaban and prednisone 80 mg daily. The dose of hydroxyurea was increased and at 2.5 g daily was effective in lowering his AEC, but led to the development of severe lower extremity skin ulcerations requiring wound care. In November 2015, hydroxyurea was discontinued and he was started on pegylated interferon-$\alpha$. The dose was slowly escalated to 180 mcg weekly with moderate control of his eosinophilia. At the time of enrollment, he complained of fatigue and pruritic skin rash on pegylated interferon-$\alpha$ 180 mcg weekly. AEC was 2,350 cells/mm$^3$. He was assigned to placebo and his first dose of benralizumab was given at week 12. At week 13, his symptoms remained stable and AEC was 110 cells/mm$^3$. Interferon-$\alpha$ was discontinued with dramatic improvement in his energy level, mood and pruritus. His course was complicated by the development of dermatomal Herpes zoster infection involving the right upper chest at week 20 that was treated with valacyclovir. At week 48, he was asymptomatic with AEC of 0 cells/mm$^3$.

**Patient 19** is a 44-year old woman with a history of asthma, and slow onset of increasing allergic disease including food reactions, hives and angioedema. She was diagnosed with idiopathic HES with cardiac, pulmonary and dermatologic manifestations in 2015 when she presented in acute heart failure and was found to have marked peripheral eosinophilia (peak AEC 11,340 cells/mm$^3$), pericarditis, myocarditis, pulmonary infiltrates/effusions, as well as a pruritic plaque-like rash. This acute event left her with a cardiac ejection fraction of 35-40%. Her symptoms improved with high dose prednisone treatment but recurred when tapering below 10 mg daily. At baseline, she reported urticarial rash, dyspnea, sinus congestion and fatigue on prednisone 7.5 mg daily. AEC was 2,830 cells/mm$^3$. She received her first dose of benralizumab on day 0 and five hours later developed a mild headache, malaise and subjective warmth without elevation in temperature. Symptoms resolved within a few hours without treatment. The following day her LDH was elevated at 254 U/L, and she noted disappearance of an erythematous rash on her chest. By day 3, she reported an improvement in her shortness of breath. AEC was 0 cells/mm$^3$ at week 13 and a prednisone taper was initiated. She was able to discontinue prednisone by week 17. At week 20, she developed a papular pruritic rash on the chest, back and extremities. Skin biopsy showed lymphocytic infiltrates without evidence of eosinophils by H&E, and the rash resolved with topical antihistamines. At week 48, she remained on benralizumab

therapy alone with an AEC of 0 cells/mm$^3$ and control of her HES symptoms. Cardiac ejection fraction was moderately improved at 46%.

**Patient 20** is a 60-year old man with a history of asthma, sinusitis, adult-onset eczema and pulmonary sarcoid who was noted to have a peripheral eosinophil count of 9,240 cells/mm$^3$ in 2014 when he was admitted for a post-operative infection after an orthopedic procedure. Beginning in 2015, his AEC began to rise further and his asthma symptoms worsened to the point that he required monthly prednisone bursts and ultimately, in December 2016, began prednisone 30 mg daily. Despite this, he continued to have coughing fits, shortness of breath, and wheezing that limited his activity and required frequent use of rescue inhalers. At baseline, his AEC was markedly elevated at 21,580 cells/mm$^3$ on prednisone 30 mg daily. He was randomized to the drug arm, and received his first dose of benralizumab without adverse event. By week 1, he reported improvement in his pulmonary symptoms. At week 13, AEC was 0 cells/mm$^3$ and a prednisone taper was initiated. Prednisone was discontinued in July 2018. His course was complicated by an episode of diverticulitis treated with levofloxacin, levofloxacin-induced tendinopathy, and mild worsening of his eczema in the setting of the prednisone taper. At week 48, he was asymptomatic with AEC 0 cells/mm$^3$ on benralizumab monotherapy.

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